

## Research Article

## The Role of Progesterone-Induced Blocking Factor in Threatened Abortion

*Peran Progesterone-Induced Blocking Factor pada Abortus Iminens*

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## Abstract

**Objective:** To determine the role of progesterone-induced blocking factor (PIBF) in women with threatened abortion.**Methods:** This was a cross-sectional study. The blood serum of two groups, the first one was women with normal gestation of  $\leq 20$  weeks, and the second one was those with imminent abortion in Prof. Dr. R.D. Kandou Hospital, and Subcenter Hospital in Manado, was collected. Samples were processed with PIBF ELISA-kit.**Results:** PIBF serum value of women in normal gestation  $\leq 20$  weeks is  $(47.153 \pm 23.830)$  ng/ml and threatened abortion is  $(11.540 \pm 4.892)$  ng/ml, with  $p$  value = 0.000.**Conclusion:** PIBF serum value of women with threatened abortion is significantly lower compared to women of normal gestation  $\leq 20$  weeks. This study showed that PIBF has an important role in maintaining pregnancy and can be used as a biologic marker of a pathologic process in pregnancy.

[Indones J Obstet Gynecol 2017; 5-4: 193-198]

**Keywords:** early pregnancy, pregnancy immunology, progesterone-induced blocking factor, threatened abortion

## Abstrak

**Tujuan:** Mengetahui kadar PIBF serum perempuan hamil usia kehamilan normal  $\leq 20$  minggu dan abortus iminens sehingga dapat menambah pemahaman mengenai PIBF sebagai petanda biologis patologi kehamilan, dan dalam upaya terapi rasional pasien dengan abortus iminens.**Metode:** Penelitian analitik komparatif potong lintang terhadap 32 pasien yang dibagi menjadi 2 kelompok kehamilan normal  $\leq 20$  minggu, dan abortus iminens di RSUP Prof Dr. R.D. Kandou, beserta RS Jejaring di wilayah Manado, dilakukan pengambilan sampel serum. Sampel dilakukan pemeriksaan kadar PIBF dengan ELISA-kit. Data diproses menggunakan program SPSS versi 22.0.**Hasil:** Kadar PIBF serum perempuan hamil usia kehamilan  $\leq 20$  minggu normal  $(47,153 \pm 23,830)$  ng/ml dan abortus iminens  $(11,540 \pm 4,892)$  ng/ml ( $p=0,000$ ).**Kesimpulan:** Kadar PIBF serum perempuan hamil dengan abortus iminens lebih rendah secara bermakna dibandingkan kadar PIBF serum perempuan hamil usia kehamilan  $\leq 20$  minggu normal. Hasil ini menunjukkan bahwa PIBF berperan dalam mempertahankan kehamilan dan dapat digunakan sebagai sarana petanda adanya proses patologi dalam suatu kehamilan.

[Maj Obstet Ginekolog Indones 2017; 5-4: 193-198]

**Kata kunci:** abortus iminens, imunologi kehamilan, kehamilan muda, progesterone-induced blocking factorCorrespondence: Andre C Widya. [widya\\_andre@yahoo.com.sg](mailto:widya_andre@yahoo.com.sg)

## INTRODUCTION

Fetus is a semi-allogenic tissue which relies inside the body of a women whose immune-competent of producing rejection immune response, therefore modulation of maternal immune response is required to maintain pregnancy.<sup>1</sup>

During pregnancy, many complications may occur, which may lead to early termination of pregnancy. Abortion is defined as the loss of pregnancy either spontaneous or induction before being viable (gestational age  $< 20$  weeks). Abortion can result in physical and emotional disturbance. The incidence of spontaneous abortion is between 15-20% from all pregnancy. Predisposing factors

of spontaneous abortion include genetic factors, anatomy, endocrine, immune, infection, thrombophilia, and idiopathic.<sup>2-4</sup>

Maternal immune response to fetus has an important role as a predisposing factor in spontaneous abortion, and it is often unexplainable. The current hypothesis is the presence of multiple factors which may affect at systemic, and local *in utero*, determine to modulate immune response into decreasing inflammation response of trophoblasts. Factors involved in this process are hormones, particularly progesterone, embryonic hormones, and Natural Killer cells (NK cells).<sup>1,2</sup>

In daily clinical practice, progesterone supplement is often used as a supportive therapy for pregnant women who are diagnosed with threatened abortion. Progesterone has an important role in modulating immune response in early trimester pregnancy through modulating pro-inflammatory and anti-inflammatory cytokines from conceptus, resulting in the continuation or termination of pregnancy.

With the influence of progesterone, lymphocyte releases protein called progesterone-induced blocking factor (PIBF), which mediates the modulation of immune response and the anti-abortion effect of progesterone. This process also gives positive feedback, there by increasing the amount of progesterone receptors in the activated lymphocyte in the placental cells and CD8<sup>+</sup> cells. In return the amount of PIBF will increase along with the gestational age from week 6 to 36, and after 41 weeks, PIBF will decrease considerably and induce the parturition. PIBF also inhibits peripheral NK cells activity. The biological effects of PIBF as mentioned above indicates that PIBF has a role in maintaining pregnancy. Therefore, PIBF value in the body liquid can predict the prognosis of pregnancy.<sup>5</sup>

A Cochrane review in 2013 demonstrated that there was no significant difference between progesterone supplementation and placebo in maintaining pregnancy. Nevertheless, Check JH mentioned that although progesterone deficiency occurs in one third of pregnancy, progesterone supplementation to improve PIBF value still has a role in decreasing 33% spontaneous abortion.<sup>6-8</sup>

Considering the impact of abortion in the physical and emotional aspects of the patient's, and to

learn further the role of immune-endocrine (PIBF) in affecting the prognosis of pregnancy, we are interested in doing this study.

## METHODS

This was a cross-sectional study. Subjects were pregnant women with gestational age of  $\leq 20$  weeks at Prof. Dr. R.D. Kandou Hospital, and subcenter hospital in Manado during the period of October 2015 to February 2016. Samples were collected by consecutive sampling. Total population consisted of 32 patients, divided into two groups: 16 with normal pregnancies, and 16 threatened abortions that matched the inclusion and exclusion criteria.

Inclusion criteria were single pregnant women with less than 20 weeks of gestation confirmed by ultrasound. Exclusion criteria were pregnant women with history of recurrent pregnancy loss, those who were currently receiving progesterone therapy, and fetal death or blighted ovum.

Blood samples from subjects were collected, and examined with PIBF ELISA-kit in the Prokita Laboratory Manado.

Data were analyzed using T-test to investigate the significance of PIBF value in the normal early pregnancy and threatened abortion. Data were processed using Statistical Product and Service Solutions (SPSS) 22.0 for Windows.

## RESULT

### Subject Characteristics

**Table 1.** Maternal Age Frequency Distribution

		Maternal age (year)			Total
		<20	20-34	$\geq 35$	
Groups	Normal pregnancy	1	10	5	16
	Threatened abortion	1	12	3	16
Total		2	22	8	32

*Interpretation: most maternal age of subjects are around 20-34 years old.*

**Table 2.** Educational Status Frequency Distribution

		Educational status				Total
		Elementary	Junior high school	High school	Bachelor	
Groups	Normal pregnancy	2	1	9	4	16
	Threatened abortion	4	6	5	1	16
Total		6	7	14	5	32

*Interpretation: most educational status of subjects are at high school.*

**Table 3.** Gravidity Status Frequency Distribution

		Gravidity status		Total
		First	Multi	
Groups	Normal pregnancy	4	12	16
	Threatened abortion	6	10	16
Total		10	22	32

*Interpretation: most gravidity status of subjects are multiple.*

**Table 4.** Gestational Age Frequency Distribution

		Gestational age (weeks)				Total
		4-8	9-12	13-16	17-20	
Groups	Normal pregnancy	7	6	3	-	16
	Threatened abortion	1	4	2	9	16
Total		8	10	5	9	32

*Interpretation: most gestational age of subjects are around 9-12 weeks.*

## PIBF Serum Value

Our study showed that PIBF serum value of threatened abortion was lower than normal pregnancy in all groups of gestational age; with details as follow: the first group of 4-8 weeks of gestation (16.114 vs 48.62 ng/ml); in the second group of 9-12 weeks of gestation (11.107 vs 41.909 ng/ml); in the third group of 13-16 weeks of gestation (7.372 vs 54.217 ng/ml); while in the last group of 17-20 weeks of gestation there was no subject in the normal pregnancy group to compare with the threatened abortion (12.151 ng/ml).

An overall calculation of our data showed a significant difference of PIBF serum value in normal pregnant women of  $\leq 20$  weeks and threatened abortion (47.153 vs 11.540 ng/ml; p value=0.000). Variable coefficient data showed that the variation of PIBF serum in both groups were above 10%, this stated that the PIBF serum were not homogenous.

## DISCUSSION

### Pregnancy immune-biology

Throughout history of human evolution, humankind has developed a mechanism to protect us from parasites and infections by detecting and destroying foreign organic materials that enter human's body. This mechanism is called the immune system.<sup>9</sup>

Fetus is a product of conception, which is a semi-allograft tissue. However, there is no rejection from the maternal immune system. This is due to the absence of MHC class I nor class II on the placental villous trophoblast, hence not inducing the NK cytotoxic activities.<sup>10,11</sup>

Another factor protecting the fetus is the expression of FasL, a protein membrane type II that is commonly present in the immune-compromised tissue, such as testes, cornea, trophoblast (Houston, and O'Connell, 2004).<sup>12</sup>

Wegmann (1993) mentioned that Th1 (pro-inflammatory) and Th2 (anti-inflammatory) balance is the key of successful pregnancy. Whereas Th2 domination is required to protect feto-placental unit from the adaptive immune response, or non-specific inflammatory response.<sup>10,12</sup>

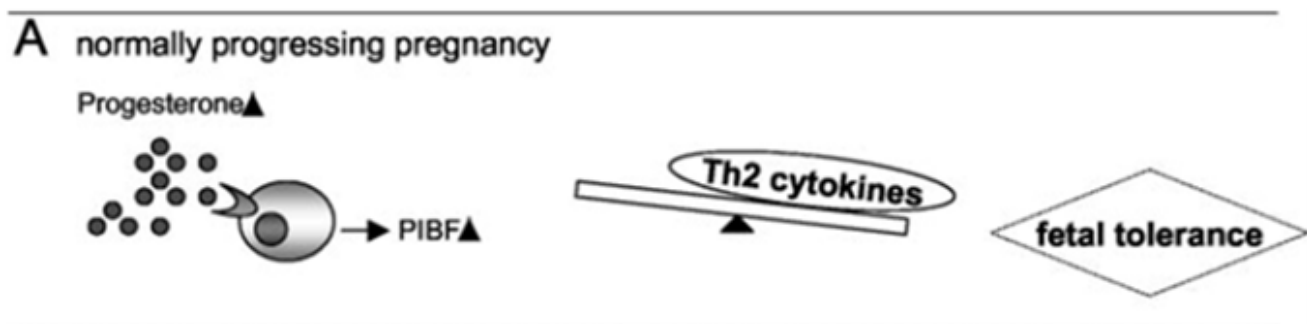
Pro-inflammatory cytokines are critical in the early process of implantation to induce angiogenesis. Shortly after, it should be replaced by anti-inflammatory cytokines that shift the balance from Th1 to Th2 dominance. Szekeres-Bartho explained that TCD8<sup>+</sup> cells in pregnant women expressed progesterone receptors since early pregnancy. Whereas, under progesterone influence, this lymphocyte cells express a mediator protein with molecular weight of 34kDa called PIBF (Progesterone-induced blocking factor).<sup>3,5,10,12,13</sup>

through mediator PIBF. Depletion of progesterone production in pregnancy will initiate parturition. This is the main reason of progesterone supplementation therapy as luteal support to prevent abortion.<sup>15</sup>

### PIBF as a research variable

As mentioned above, to have an immune-endocrine effect, progesterone needs protein mediator which is PIBF, which synthesized from the activated lymphocyte of T<sub>γ/δ</sub> (CD8<sup>+</sup>).<sup>1-3,5,7</sup> Progesterone supplementation is meant to improve PIBF value through its binding to progesterone receptors, and not to improve progesterone level.<sup>3-7,16</sup>

The mechanisms of PIBF in maintaining pregnancy are through several pathways: by inhibiting peripheral NK cells cytotoxicity activity, by inducing Th2 cytokines domination and asymmetric antibody production (Ig G).<sup>17-22</sup>



**Figure 1.** Normally Progressing Pregnancy. A schematic hypothesis of immune-endocrinology in pregnancy. In normal pregnancy, a sufficient concentration of progesterone leads to anti-inflammatory cytokines dominance (Th2), mediated by PIBF expression.<sup>13</sup>

### The role of NK cells in pregnancy

NK cells dominated the leucocytes involved in the implantation process in early pregnancy. NK cells are classified as peripheral NK cells and uterine NK cells. In pregnancy, the amount of peripheral NK cells is depleted due to the minimal expression of CD16. This process is affected by the progesterone level.<sup>14</sup> In contrast to peripheral NK cells, uterine NK cells are dominated in pregnancy. These NK cells have a small effect of cytotoxicity hence needed in pregnancy.<sup>14</sup>

### Role of progesterone in pregnancy

Progesterone is the main hormone in maintaining pregnancy. It directly affects immune system or

PIBF expression required adequate, hence PIBF value will predicts the outcome of pregnancy. This is according to the study done by B Polgar, etc which stated that PIBF in the body liquid will reflect the pathological condition of pregnancy.<sup>1,2,5,21,23</sup>

PIBF value can be measured through urine or serum sample, this is due to the fact that the weight molecule is 34kDa, there by small enough to be excreted by renal of PIBF, this could be detected in the urine. PIBF value measurement through urine sample is often done because it is non invasive, yet 24 hours urine sample is needed to reduce the bias.<sup>1-3,5</sup> Nonetheless, in this study we use serum sample, because it is considered the more consistent result due to a random one time sampling.

A study performed by Igor Hudic found difference between PIBF value in serum and urine, nonetheless it has the same predictive value where PIBF value in women with threatened abortion is lower than in normal pregnancy.<sup>2</sup> This is consistent with our study which found that PIBF serum value in threatened abortion is significantly lower than in normal pregnancy.

### PIBF Serum Value

Previous studies have shown that PIBF has a role in maintaining pregnancy through immunological mechanism by modulating cytokine Th1/Th2 balance, suppressing cytotoxic and cytotoxic activity of NK cells, and by increasing asymmetric antibody production.<sup>1-3,5,7,13,19</sup>

Our study showed that there were significant difference between PIBF serum value in normal pregnant women  $\leq 20$  weeks ( $47.153 \pm 23.830$ ) and threatened abortion ( $11.540 \pm 4.892$ ) ( $p$  value=0.000). This result is consistent with several studies which suggested that the presence of PIBF could be used as a biomarker of predicting the outcome of pregnancy or detecting pathological condition of pregnancy.<sup>1,2,5</sup>

Our study showed that the coefficient variation is above 10%, meaning that PIBF value has a large variation. This might be due to the factors beyond the researcher control which cause the difference of progesterone production such as inflammatory process, different fetal development, infection factor, uterine stretch, or the maternal/fetal stress.<sup>24</sup> Similar studies performed before also have the same conclusion where mean PIBF in threatened abortion is lower significantly compared to normal pregnancy. Despite having different value, this may due to the different study methods, reagents, processing methods, wave length used in ELISA reader, samples amount, and gestational age used in the subjects.

This study showed that PIBF could be used as a biomarker of pathologic process in pregnancy. However, due to the cross-sectional study design, it is not possible to determine the cut-off point of PIBF value required to maintain pregnancy.

### CONCLUSIONS

PIBF serum value of pregnant women  $\leq 20$  weeks with threatened abortion were significantly lower

compared to normal pregnancy. This also showed that PIBF value could be used as a biomarker of a pathologic process in pregnancy. PIBF could also be used as a rational therapy in threatened abortion. Further prospective studies are required to determine the cut-off point of PIBF value necessary to maintain pregnancy.

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